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Temporal Expression of the *Bacillus subtilis* *secA* Gene, Encoding a Central Component of the Preprotein Translocase

MARKUS HERBORT,¹ MICHAEL KLEIN,¹ ERIK H. MANTING,² ARNOLD J. M. DRIESSEN,²
AND ROLAND FREUDL^{1*}

*Institut für Biotechnologie 1, Forschungszentrum Jülich GmbH, D-52425 Jülich, Germany,¹ and
Department of Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute,
University of Groningen, Kerklaan 30, NL-9751 NN Haren, The Netherlands²*

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In *Bacillus subtilis*, the secretion of extracellular proteins strongly increases upon transition from exponential growth to the stationary growth phase. It is not known whether the amounts of some or all components of the protein translocation apparatus are concomitantly increased in relation to the increased export activity. In this study, we analyzed the transcriptional organization and temporal expression of the *secA* gene, encoding a central component of the *B. subtilis* preprotein translocase. We found that *secA* and the downstream gene (*prfB*) constitute an operon that is transcribed from a vegetative (σ^A -dependent) promoter located upstream of *secA*. Furthermore, using different independent methods, we found that *secA* expression occurred mainly in the exponential growth phase, reaching a maximal value almost precisely at the transition from exponential growth to the stationary growth phase. Following to this maximum, the de novo transcription of *secA* sharply decreased to a low basal level. Since at the time of maximal *secA* transcription the secretion activity of *B. subtilis* strongly increases, our results clearly demonstrate that the expression of at least one of the central components of the *B. subtilis* protein export apparatus is adapted to the increased demand for protein secretion. Possible mechanistic consequences are discussed.

Transport of secretory proteins across the *Bacillus subtilis* plasma membrane is mediated by an export machinery which consists (at least partially) of components that are homologous to the well-characterized Sec proteins of *Escherichia coli* (52). Genes encoding homologues of the inner membrane proteins SecY (27, 43) and SecE (15), which most likely form the core of the protein-conducting pathway (8), and SecDF (3), which is thought to increase the efficiency of protein translocation at the SecYE core complex (9), have been identified in *B. subtilis*. In addition, a homologue of the *E. coli* SecA protein, which is known to couple the energy of ATP binding and hydrolysis to protein translocation (10, 20), has also been found in *B. subtilis* (31, 34). An involvement of these Sec proteins in the secretion process of *B. subtilis* has been inferred from various functional analyses (3, 15, 17, 22, 28, 31, 47, 50).

B. subtilis secretes most of its extracellular enzymes in a temporally controlled manner. Whereas during exponential growth relatively small quantities of exoproteins are released into the growth medium, a substantial increase in exoprotein secretion is observed when the cells enter the post-exponential growth phase (for reviews, see references 11 and 33). This increase in secretion activity is due mainly to an increased synthesis of the corresponding secreted proteins for which, among other regulatory networks, the DegS-DegU two-component system is of crucial importance (11, 26). However, no data are available concerning the temporal regulation of the *sec* genes during growth of *B. subtilis*. In particular, it is not known whether the amounts of some or all components of the protein translocation machinery are concomitantly increased with the temporal demand for an increased secretion activity in the post-exponential growth phase.

In the work described here, we analyzed the transcriptional organization and temporal expression of the *B. subtilis* *secA* gene, encoding the translocation ATPase subunit of the preprotein translocase. We show that *secA* is the first gene in an operon and that transcription of this operon is initiated from a promoter which is highly similar to σ^A -dependent promoters. Furthermore, we found by different independent criteria that expression of *secA* is maximal at the end of the exponential growth phase, after which a decrease in transcription can be observed. The results indicate that, like the synthesis of secreted proteins, the synthesis of at least one of the central components of the *B. subtilis* preprotein translocase is temporally controlled.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *E. coli* JM109 (53) was grown at 37°C in Luria-Bertani (LB) medium (25) containing ampicillin (100 μ g ml⁻¹) as required. *B. subtilis* DB104, a strain that secretes only low levels of proteases into the culture supernatant (16), and its derivatives DB104-4 and DB104-4T were grown at 37°C in LB, 2xYT (25), sporulation (39), or minimal (41) medium containing chloramphenicol (5 μ g ml⁻¹) or kanamycin (20 μ g ml⁻¹) as required. *B. subtilis* DB104-4, containing a transcriptional *secA-lacZ* fusion integrated into the chromosomal *amyE* gene, was constructed by transforming DB104 with linearized plasmid pDG268-4 (see below), selecting for chloramphenicol resistance and screening for an α -amylase-negative phenotype on LB plates containing 0.6% (wt/vol) amylopectin-azure (Sigma, Deisenhofen, Germany). DB104-4T, containing a translational *secA-lacZ* fusion integrated into the *amyE* gene, was constructed by the same procedure except that plasmid pDG268-4T (see below) was used.

DNA techniques and plasmid constructions. Standard procedures (36) were used for preparation of plasmid DNA, isolation of DNA fragments, restriction, ligation, and other DNA techniques.

pDG268 is a transcriptional *spoVG-lacZ* fusion vector that allows the subsequent integration of the respective *lacZ* reporter fusions in single copy into the chromosomal *amyE* gene of *B. subtilis* via a double-cross-over event (1). A transcriptional *secA-lacZ* fusion was constructed by ligating a 456-bp *MscI/HindIII* fragment, containing the *orf189-secA* intergenic region and the 5' end of the *secA* gene, from plasmid pBO1 (31) into pDG268 which had been digested with *EcoRI*, filled in with Klenow DNA polymerase, and then redigested with *HindIII*. In the resulting plasmid, pDG268-4, transcription of the *spoVG-lacZ* reporter gene is under the control of the *secA* promoter. For construction of a *secA-lacZ*

* Corresponding author. Mailing address: Institut für Biotechnologie 1, Forschungszentrum Jülich GmbH, D-52425 Jülich, Germany. Phone: (49) 2461-613472. Fax: (49) 2461-612710. E-mail: r.freudl@fzjuelich.de.

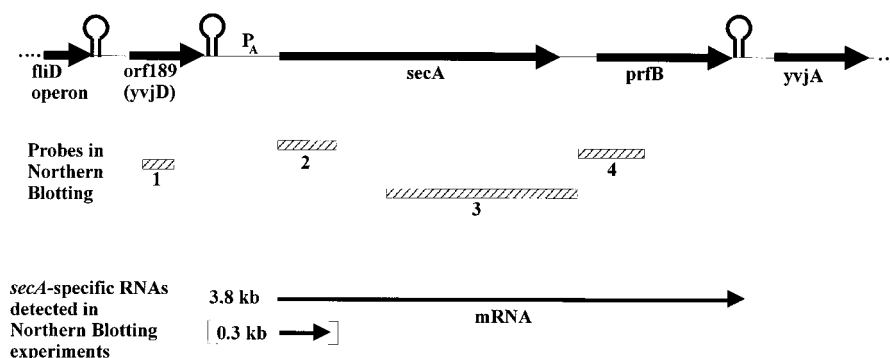


FIG. 1. Gene organization in the *secA* region of the *B. subtilis* chromosome and positions of *secA*-specific transcripts. P_A , σ^A -dependent promoter of the *secA-prfB* operon. Stem-loop figures, putative rho-independent terminators; numbered bars, locations of hybridization probes used in Northern blotting experiments; thin arrows, *secA*-specific transcripts identified in the Northern hybridization experiments.

translational fusion, a 892-bp *HindIII/ClaI* fragment of pDG268-4, encompassing the *spoVG-lacZ* translational initiation signals (i.e., ribosome-binding site and start codon) was replaced by a 846-bp *HindIII/ClaI* fragment from the translational *lacZ* fusion vector pRB381 (5). To obtain an in-frame fusion, both *HindIII* ends were filled in with Klenow DNA polymerase. The resulting plasmid, pDG268-4T, encodes a fusion protein of 65 amino-terminal amino acid residues of *SecA* fused to the β -galactosidase reporter, and transcription of the corresponding gene is under the control of the *secA* promoter. For integration of the *secA-lacZ* fusions into the *amyE* gene, pDG268-4 and pDG268-4T were linearized with *MscI* and used to transform *B. subtilis* DB104. Chloramphenicol-resistant and amylase-negative transformants were selected, resulting in *B. subtilis* DB104-4 and DB104-4T, respectively.

For generation of the digoxigenin (DIG)-labeled RNA probes 1 to 4 (Fig. 1), suitable DNA fragments of the *orf189-secA-prfB* region were cloned into the riboprobe vector pGEM3Z (Promega, Mannheim, Germany). For probe 1, covering an internal fragment of *orf189*, a 290-bp *MscI/BglII* fragment from plasmid pBO1 was cloned into *BamHI/HindIII*-digested pGEM3Z, resulting in plasmid pMHP1. For probe 3, covering the 3' end of *secA*, a 2.63-kb *PstI* fragment from plasmid pMKL4 (17), encompassing the entire *secA* gene, was ligated into *PstI*-digested pGEM3Z. Before in vitro transcription from the SP6 promoter was initiated, the resulting plasmid pMHP3 was linearized at a *ClaI* site, located within the *secA* coding region, thereby resulting in a DIG-labeled RNA probe derived solely from the 3' end of the *secA* gene. For probe 2, covering the 5' end of *secA*, a 448-bp *BamHI/HpaI* fragment from pMHP3 was ligated into *BamHI/HindIII*-digested pGEM3Z, resulting in plasmid pMHP2. For probe 4, covering part of the intergenic *secA-prfB* region and the 5' end of the *prfB* gene, a 488-bp *Asp718/PstI* fragment from pMKL4 was ligated into *Asp718/PstI*-digested pGEM3Z, resulting in plasmid pMHP4.

RNA techniques. To isolate total RNA, we used an RNeasy total RNA kit (Qiagen, Hilden, Germany) as instructed by the manufacturer. The amount of total RNA was quantified by measuring the optical density at 260 nm (OD_{260}) of each preparation. Furthermore, the RNA samples used in the Northern blotting and the primer extension experiments were additionally examined with respect to the amount and intactness of the RNA preparations by formaldehyde-agarose gel electrophoresis and staining with ethidium bromide (49). Northern blot analyses were performed essentially as described by Börmann et al. (4). To obtain DIG-labeled RNA probes 1 to 4 (locations in the *orf189-secA-prfB* region are shown in Fig. 1), plasmids pMHP1 to -4 were used as templates for in vitro transcription using a DIG RNA labeling kit (Boehringer Mannheim GmbH, Mannheim, Germany) as instructed by the manufacturer. For detection of hybridization signals, a Boehringer nonradioactive nucleic acid labeling and detection kit was used. Primer extension experiments were performed as described by Sambrook et al. (36). The synthetic oligonucleotide OMKL48 (GTATCTATTC AGCGTACGT), which is complementary to the noncoding strand of the 5' end of the *secA* gene (corresponding to nucleotides 39 to 57 of the *secA* structural gene), was used as the primer in the primer extension experiments. Dideoxynucleotide chain termination sequencing reactions (37), using the same primer and an appropriate plasmid DNA (pBO1) as the template, were run in parallel on the gel to allow determination of the endpoints of the extension products.

Other techniques. β -Galactosidase activities of *B. subtilis* cells containing transcriptional or translational *secA-lacZ* fusions were determined by the procedure of Nicholson and Setlow (29), using *o*-nitrophenyl- β -D-galactopyranoside as the substrate. The measured β -galactosidase activity was normalized by the method of Miller (25). α -Amylase secreted into the culture supernatant of *B. subtilis* DB104 was assayed by the method of Bernfeld (2), and the resulting activities (units) were expressed in terms of micromoles of maltose liberated in 60 min at 37°C by 1 ml of culture supernatant. Total amounts of secreted proteins in the culture supernatant of DB104 were determined by the bicinchoninic acid method of Walker (51) after precipitation of the proteins with trichloroacetic acid (10%,

final concentration). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting using anti-*B. subtilis* *SecA* antibodies were performed as described previously (24).

RESULTS

The *B. subtilis* *secA* gene is the first gene in a bicistronic operon. The chromosomal organization of the *B. subtilis* *secA* region is shown in Fig. 1. The *secA* gene is located downstream of the *fliD* operon, whose gene products are involved in the formation of flagella, and a gene (*orf189*) of unknown function, which is followed by a putative rho-independent terminator (7). Downstream of *secA*, we identified a gene (*prfB*) which is preceded by a putative ribosome-binding site and which encodes a homologue of the *E. coli* protein release factor 2 (32). No putative promoter or terminator sequences are found in the *secA-prfB* intergenic region, suggesting that *secA* and *prfB* may be located in an operon which is transcribed by a promoter located upstream of *secA*. In fact, a promoter sequence (TTGGAA-16 bp-TATGAT) showing good agreement to the consensus promoter sequence recognized by the major vegetative sigma factor (σ^A) (12) was identified 80 to 107 bp upstream of the *secA* start codon (31, 34). In addition, an operon structure consisting of *secA* and *prfB* was also suggested from computer analyses of the complete nucleotide sequence of the *B. subtilis* chromosome (18).

For identification of *secA*-specific transcripts, total RNA was extracted from *B. subtilis* DB104 at various time points during growth (measured by OD_{600}) in sporulation medium (Fig. 2A), and Northern hybridization experiments were performed with DIG-labeled RNA probes, encompassing the 3' end of *orf189* (probe 1), the 5' end of *secA* (probe 2), the 3' end of *secA* (probe 3), and the 5' end of *prfB* (probe 4) (Fig. 1). With the 5' end of *secA* as a probe, two major hybridizing bands, 3.8 and 0.3 kb in size, were identified (Fig. 2B). Furthermore, additional hybridization signals were found associated with the 16S and 23S RNAs; these most likely were caused by hybridization to degradation products which were trapped by the rRNAs (14). The 3.8-kb mRNA was also detectable with probes 3 and 4 but not with probe 1 (data not shown), strongly suggesting that *secA* and *prfB* are transcribed together starting from a promoter located in the *orf189-secA* intergenic region. The 0.3-kb RNA could be detected with probe 2 only (not with probes 1, 3, and 4 [data not shown]), suggesting that this RNA might represent a stable breakdown product from the 5' end of the larger 3.8-kb *secA-prfB* transcript or, alternatively, might be produced by premature transcription termination early in the *secA* gene. A further observation was that the amount of de-

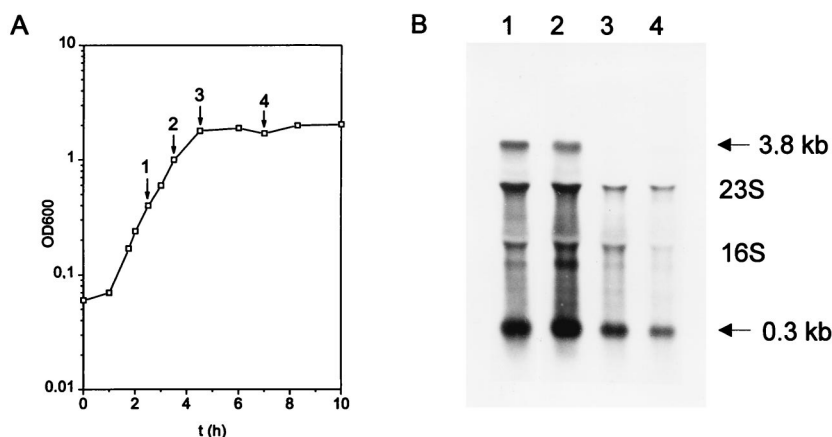


FIG. 2. Northern blot analysis. (A) Growth of *B. subtilis* DB104 in sporulation medium. Samples 1 to 4 were withdrawn for total RNA isolation at the time points indicated. (B) Northern hybridization of equal amounts of total RNA isolated from samples 1 to 4 (lane numbers correspond to sample numbers), using a probe (probe 2; Fig. 1) derived from the 5' end of the *secA* gene. Positions of the *secA*-specific RNAs are indicated by arrows. 16S and 23S denote positions of the 16S and 23S rRNAs, respectively.

tectable *secA*-specific transcripts in the RNA preparations isolated from cells grown to the end of the exponential (lane 3) or to the post-exponential phase (lane 4) was significantly lower than the corresponding signals which were obtained with RNA isolated from exponentially growing cells (lanes 1 and 2), suggesting that *secA* transcription is maximal during vegetative growth.

To identify the start point of the *secA-prfB*-specific transcript, primer extension experiments were performed with primer OMKL48, using RNA isolated at various time points during growth in rich (2xYT) medium. As shown in Fig. 3, a signal corresponding to a transcriptional start site located 73 bp upstream of the *secA* translational start codon was detected in all samples. Since this transcriptional start site is located imme-

diately downstream to the σ^A -dependent promoter previously identified by sequence analysis, our data clearly indicate that this promoter is in fact the promoter element involved in *secA* transcription initiation. We observed that in the primer extension experiments, as in the Northern blotting experiments, the intensities of the obtained signals were significantly higher in RNA preparations extracted from exponentially growing cells (lanes 1 and 2) than in RNA extracted from cells grown to the late exponential (lane 3) or post-exponential (lane 4) phase. Identical results were obtained for RNA isolated at comparable time points during growth from cells that had been grown in sporulation medium (data not shown).

Transcription of the *secA* gene is temporally regulated. Since the results described above suggested that expression of *secA* is

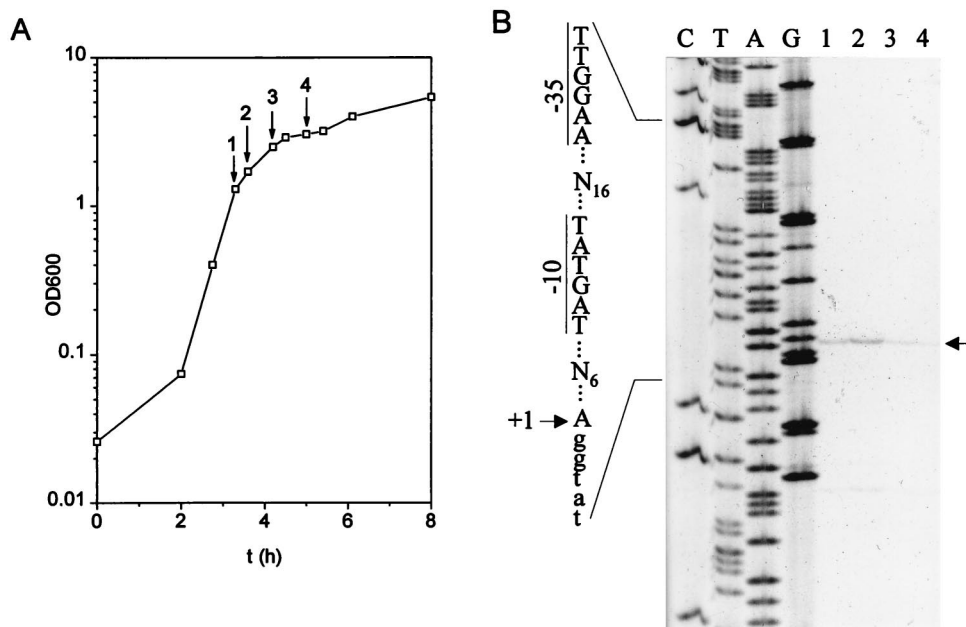


FIG. 3. Mapping of the 5' end of the *secA-prfB* mRNA by primer extension. (A) Growth of *B. subtilis* DB104 in 2xYT medium. Samples 1 to 4 were withdrawn for total RNA isolation at the time points indicated in the growth curve. (B) Primer extension experiment using oligonucleotide OMKL48 as the primer and equal amounts of RNA isolated from samples 1 to 4 (lane numbers correspond to sample numbers). The major extension product is indicated by an arrow. Lanes C, T, A, and G, sequencing ladder obtained with the same primer (OMKL48) and pBO1 (31) as the template. The relevant part of the nucleotide sequence is shown on the left.

temporally modulated in the cell, quantitative measurements of *secA* expression were performed with transcriptional and translational fusions of the *lacZ* reporter gene to 5' fragments of *secA*. A 456-bp *MscI/HindIII* DNA fragment from pBO1 (31) containing the *orf189-secA* intergenic region and the 5' end of the *secA* gene was cloned into the *lacZ* fusion vector pDG268 (1) in two different ways. In plasmid pDG268-4, the *spoVG-lacZ* gene of pDG268 was placed under the regulatory control of the *secA* promoter, resulting in a transcriptional fusion. In pDG268-4T, the 5' end of *secA* was fused in frame to the *lacZ* reporter gene, resulting in a translational *secA-lacZ* fusion. Both fusions were introduced by double-crossover recombination in single copy into the *amyE* locus of the chromosome of *B. subtilis* DB104, and β -galactosidase activity in the resulting strains (DB104-4 and DB104-4T) was monitored throughout growth (Fig. 4). In both strains, an increase in β -galactosidase activity was observed during exponential growth, reaching a maximal value at the end of the exponential growth phase (which is defined as T_0). Upon entering the post-exponential phase, the strains exhibited a relatively sharp decrease of β -galactosidase activity, reaching a low basal level approximately 2 to 3 h after T_0 . The observed patterns were independent of the growth medium, since similar profiles of β -galactosidase activity were obtained with both strains in sporulation medium (Fig. 4A and C), rich medium (Fig. 4B and D), and minimal medium (data not shown). In addition, these results were in agreement with the temporal effects observed in the Northern blotting and primer extension experiments, which also indicated a maximal expression of *secA* during exponential growth and a sharp decrease of transcription shortly after T_0 . Furthermore, the analysis of α -amylase secretion (Fig. 4E) and of protein secretion in general (by measuring the amount of total protein in the supernatant) (Fig. 4F) during growth showed that the time point of maximal *secA* expression coincides with the beginning of the high-level protein secretion period in *B. subtilis*.

Next, expression of *secA* was analyzed by Western blotting using SecA-specific antibodies. Cells of *B. subtilis* DB104 were grown in sporulation medium (Fig. 5A and C) or LB medium (Fig. 5B and D), and samples were taken at various time points during growth. Equal amounts of proteins were applied to SDS-PAGE followed by Western blotting. In both media, the amount of SecA protein reaches a maximal value around T_0 , decreasing slowly thereafter to significantly lower levels. These results are consistent with those obtained with the *lacZ* reporter gene fusions, indicating that *secA* expression is strongly decreased at T_0 and that the SecA protein synthesized up to this point is diluted within the cells by ongoing cell division. Our finding that SecA protein can nevertheless be detected several hours after the end of exponential growth can be explained by the high in vivo stability of the *B. subtilis* SecA protein (46) and eventually by some residual de novo transcription of the *secA* gene.

DISCUSSION

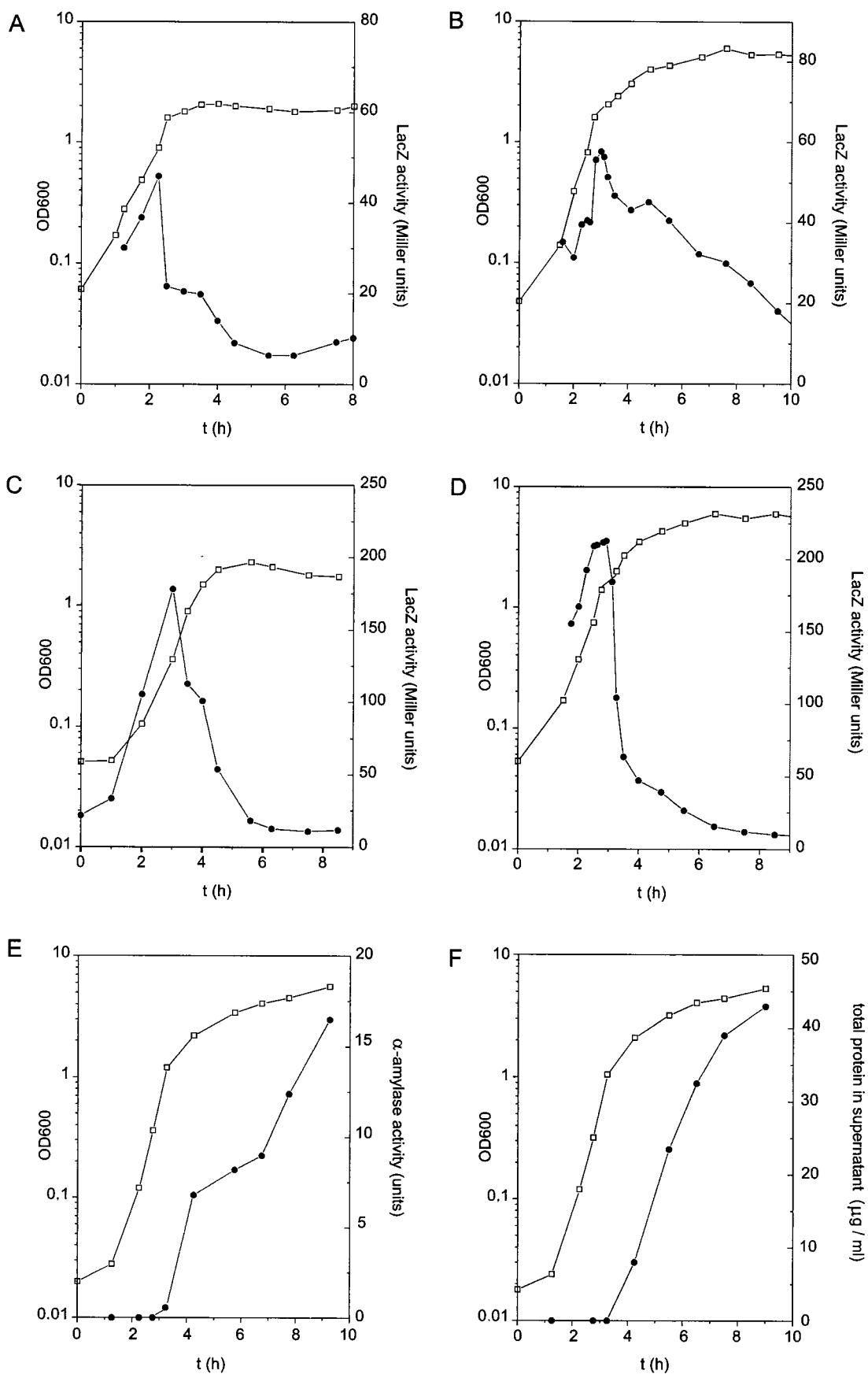
The increase in protein secretion activity is, among competence development and sporulation, one of the various possible post-exponential growth means by which *B. subtilis* and related

Bacillus species adapt to unfavorable growth conditions, such as nutrient limitation (42). Synthesis of the precursor forms of the secreted proteins drastically increases at the level of transcription of the corresponding genes upon transition from exponential to stationary growth, and this increase is controlled by complex regulatory networks (11). It remains an open question whether the number of protein export sites normally present in low-level secreting exponentially growing cells is sufficient to allow the effective translocation of the abundance of exoproteins in the postexponential growth phase or whether the amounts of one or several components of the protein secretion apparatus must be adapted to this elevated secretion activity.

In this report, we have shown that the *secA* gene, encoding one of the central components of the *B. subtilis* preprotein translocase, is transcribed predominantly in the exponential growth phase. This result is in agreement with our finding that the *secA* gene, together with the *prfB* gene which is located downstream of *secA*, is transcribed from a promoter with a sequence very similar to promoter sequences recognized by σ^A , the major vegetative sigma factor of *B. subtilis* (12). Maximum *secA* expression is reached at the end of the exponential growth phase, almost precisely at the time point corresponding to T_0 . Surprisingly, upon transition to the stationary growth phase, de novo transcription of the *secA* gene sharply decreases to a low basal level. Despite this fact, significant amounts of SecA protein can be detected also at much later time points of growth, probably due to the long half-life of the SecA protein in *B. subtilis* (46). Upon abrupt shutoff of *secA* transcription at the transition to stationary growth phase, the slow decrease in the amount of SecA protein that is observed at later time points can be explained by a dilution of the presynthesized SecA protein to the daughter cells during the remainder of cell divisions after T_0 ; possibly some residual de novo synthesis may also occur. With respect to the time period of high-level protein secretion, which extends from approximately T_0 to T_4/T_5 (33, 38), it is worth emphasizing that maximal *secA* gene expression and, as a consequence, the highest amount of SecA protein is reached at the beginning of the period of elevated secretion activity. This implies that the amount of SecA protein synthesized up to this point is sufficient to ensure effective protein translocation during the entire high-level secretion period. Besides its role as a membrane-associated energy-coupling subunit of preprotein translocase, SecA can most likely in its soluble form (6, 22), interact also with precursor proteins occurring free in the cytosol (45). One might speculate that the high amount of SecA at the beginning of the high-level secretion period can function as a kind of a secretory protein-specific chaperone buffer which helps to maintain the massive amounts of precursor proteins in an export-competent state for their subsequent membrane translocation.

In *E. coli*, *secA* expression is coupled to the secretion status of the cell by an autoregulatory mechanism (30). Under normal protein export conditions, excess SecA binds to its own mRNA, thereby autorepressing its translation. Under conditions of limiting protein export, the mRNA-bound SecA is released (possibly by a titration mechanism) and translation of *secA* mRNA increases 10- to 20-fold (23, 35, 40). So far, we have found no evidence for a comparable autoregulation of

FIG. 4. Monitoring of *secA* gene expression during growth of cells containing *secA-lacZ* fusions and analysis of α -amylase secretion and protein secretion activity in general. Graphs show expression of a transcriptional *secA-lacZ* fusion during growth of *B. subtilis* DB104 in sporulation medium (A) or LB medium (B); expression of a translational *secA-lacZ* fusion during growth of *B. subtilis* DB104 in sporulation medium (C) or LB medium (D); secretion of α -amylase during growth of *B. subtilis* DB104 in LB medium (E); and total amount of proteins secreted into the supernatant of *B. subtilis* DB104 during growth in LB medium (F). Squares, OD₆₀₀; circles, β -galactosidase (*LacZ*) specific activity (A to D); α -amylase activity (E), or total amount of protein in the supernatant (F).



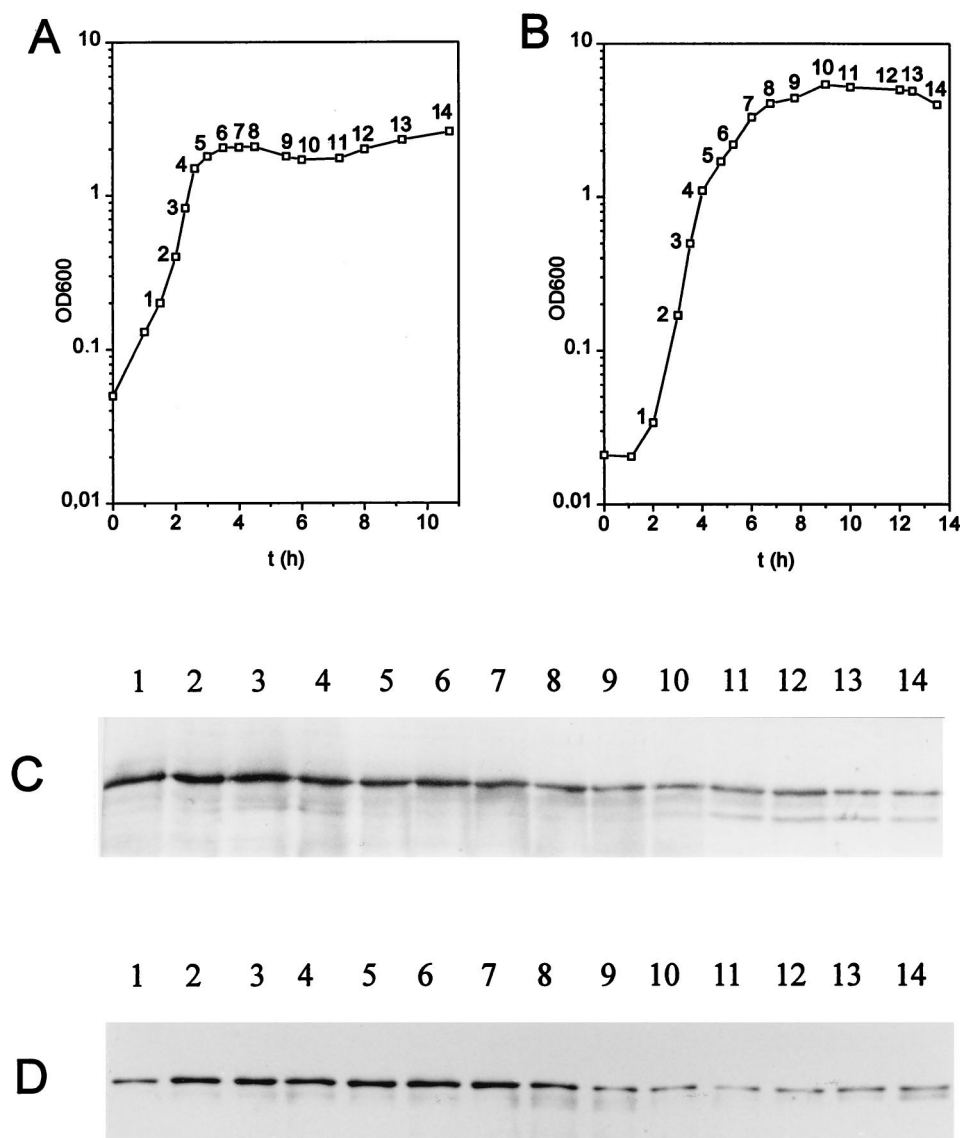


FIG. 5. Detection of SecA protein by Western blotting. (A and B) Growth of *B. subtilis* DB104 in sporulation medium (A) or LB medium (B). Samples 1 to 14 were withdrawn for the preparation of total-cell extracts at the time points indicated, and equal amounts of protein were subsequently applied to SDS-PAGE and Western blotting. (C and D) Western blot analyses using SecA-specific antibodies of total-cell extracts isolated from *B. subtilis* DB104 grown in sporulation medium (C) or LB medium (D). The lane numbers in panels C and D correspond to sample numbers in panels A and B, respectively.

secA expression in *B. subtilis*. High-level expression of a secretory protein (α -amylase AmyL of *B. licheniformis*) in *B. subtilis*, resulting in the massive accumulation of AmyL precursor in the cytosol, did not lead to an increase in the amount of SecA protein (13). Furthermore, overexpression of SecA in *B. subtilis* DB104, containing a translational *orf189-secA-lacZ* fusion integrated in the *amy* locus, did not result in a reduced expression of β -galactosidase activity (13). However, since these observations are negative evidence, the existence of an autoregulatory mechanism for *secA* expression in *B. subtilis* cannot be totally excluded.

Very little is known about the regulation of other components of the *B. subtilis* preprotein translocase. Li et al. (19) have shown that the *B. subtilis* *secY* gene, which is located within the *S10-spc- α* region and encodes one of the central integral membrane components of the translocase, is primarily cotranscribed within one large (15-kb) transcriptional unit to-

gether with the other (mostly ribosomal) genes of the *S10-spc- α* region and that this transcription is driven by two σ^A -dependent promoters located upstream of the *S10* gene. In addition, Suh et al. (44) identified two weak promoter-active regions located within the two genes immediately upstream of *secY*, which are able to stimulate transcription of a *lacZ* reporter gene mainly in the stationary growth phase. However, it is unclear whether these weak promoter sequences significantly contribute to *secY* gene expression in the stationary growth phase. Furthermore, since stable integration of SecY into the cytoplasmic membrane, at least in *E. coli*, requires a concomitant coexpression of SecE (21), it is also unclear whether the weak transcriptional activity of these potential *secY* promoter sequences in the stationary growth phase would in fact result in a significantly elevated level of SecY protein in the plasma membrane.

Recently, Bolhuis et al. cloned the *secDF* gene of *B. subtilis*

and analyzed its expression during growth in different media, using a transcriptional *secDF-lacZ* fusion (3). Whereas *secDF* expression was more or less constitutive when the cells were grown in minimal medium, a maximum of *secDF* transcription was found in the early post-exponential growth phase when rich (TY) medium was used for growth. Interestingly, the time point of maximum *secDF* expression in rich medium is 1 to 2 h later in the growth phase compared to the time point of maximum *secA* expression.

B. subtilis contains at least four closely related type I signal peptidases (SipS, SipT, SipU, and SipV) which are responsible for the removal of the signal peptides from nonlipoprotein precursor proteins (48). Whereas the *sipU* and *sipV* genes are transcribed at a more or less constant level during all growth phases, the transcription of *sipS* and *sipT* increases upon transition from exponential to stationary growth. Due to their finding that the increase in *sipS* and *sipT* transcription is controlled by the DegU-DegS two-component signal transduction system, which is mainly involved in the upregulation of the synthesis of secreted precursor proteins after T_0 , the authors (48) speculate that SipS and SipT serve to increase the capacity of *B. subtilis* for protein secretion concomitantly with the increasing amounts of secretory precursor proteins synthesized in the post-exponential growth phase. Interestingly, in contrast to *secA* gene expression, which is maximal at the beginning of the secretion period (approximately T_0), *sipS* and *sipT* transcription is relatively low at T_0 and increases steadily throughout the entire period of high-level protein secretion after T_0 (48). These findings taken together suggest that at the post-exponential growth phase, *B. subtilis* adjusts the amount of some of the central components of the protein secretion machinery in relation to the increased demand for protein export. However, differences in the timing of this adjustment seem to exist between different components.

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REFERENCES

- Antonieński, C., B. Savelli, and P. Stragier. 1990. The *spoIII* gene, which regulates early developmental steps in *Bacillus subtilis*, belongs to a class of environmentally responsive genes. *J. Bacteriol.* **172**:86–93.
- Bernfeld, P. 1955. Amylases, α and β . *Methods Enzymol.* **1**:149–158.
- Bolhuis, A., C. P. Broekhuizen, A. Sorokin, M. L. van Roosmalen, G. Venema, S. Bron, W. J. Quax, and J. M. van Dijl. 1998. SecDF of *Bacillus subtilis*, a molecular siamese twin required for the efficient secretion of proteins. *J. Biol. Chem.* **273**:21217–21224.
- Börmann, E. R., B. J. Eikmanns, and H. Sahm. 1992. Molecular analysis of the *Corynebacterium glutamicum* *gdh* gene encoding glutamate dehydrogenase. *Mol. Microbiol.* **6**:317–326.
- Brückner, R. 1992. A series of shuttle vectors for *Bacillus subtilis* and *Escherichia coli*. *Gene* **122**:187–192.
- Cabelli, R. J., K. M. Dolan, L. Qian, and D. B. Oliver. 1991. Characterization of membrane-associated and soluble states of SecA protein from wild-type and *secA51(TS)* mutant strains of *Escherichia coli*. *J. Biol. Chem.* **266**:24420–24427.
- Chen, L., and J. D. Helmann. 1994. The *Bacillus subtilis* σ^D -dependent operon encoding the flagellar proteins FliD, FliS, and FliT. *J. Bacteriol.* **176**:3093–3101.
- Duong, F., and W. Wickner. 1997. Distinct catalytic roles of the SecYE, SecG and SecDFyajC subunits of preprotein translocase holoenzyme. *EMBO J.* **16**:2756–2768.
- Duong, F., and W. Wickner. 1997. The SecDFyajC domain of preprotein translocase controls preprotein movement by regulating SecA membrane cycling. *EMBO J.* **16**:4871–4879.
- Economou, A., and W. Wickner. 1994. SecA promotes preprotein translocation by undergoing ATP-driven cycles of membrane insertion and deinsertion. *Cell* **78**:835–843.
- Ferrari, E., A. S. Jarnagin, and B. F. Schmidt. 1993. Commercial production of extracellular enzymes, p. 917–937. In A. L. Sonenshein, J. A. Hoch, and R. Losick (ed.), *Bacillus subtilis* and other gram-positive bacteria. American Society for Microbiology, Washington, D.C.
- Helman, J. D. 1995. Compilation and analysis of *Bacillus subtilis* σ^A -dependent promoter sequences: evidence for extended contact between RNA polymerase and upstream promoter DNA. *Nucleic Acids Res.* **23**:2351–2360.
- Herbert, M., and R. Freudl. Unpublished observations.
- Homuth, G., S. Masuda, A. Mogk, Y. Kobayashi, and W. Schumann. 1997. The *dnaK* operon of *Bacillus subtilis* is heptacistronic. *J. Bacteriol.* **179**:1153–1164.
- Jeong, S. M., H. Yoshikawa, and H. Takahashi. 1993. Isolation and characterization of the *secE* homologue gene of *Bacillus subtilis*. *Mol. Microbiol.* **10**:133–142.
- Kawamura, F., and R. H. Doi. 1984. Construction of a *Bacillus subtilis* double mutant deficient in extracellular alkaline and neutral proteases. *J. Bacteriol.* **160**:442–444.
- Klose, M., K.-L. Schimz, J. van der Wolk, A. J. M. Driessen, and R. Freudl. 1993. Lysine 106 of the putative catalytic ATP-binding site of the *Bacillus subtilis* SecA protein is required for functional complementation of *Escherichia coli* *secA* mutants *in vivo*. *J. Biol. Chem.* **268**:4504–4510.
- Kunst, F., N. Ogasawara, I. Moszer, A. M. Albertini, G. Alloni, V. Azevedo, M. G. Bertero, P. Bessières, A. Bolotin, S. Bochert, R. Borriss, L. Boursier, A. Brans, M. Braun, S. C. Brignell, S. Bron, S. Brouillet, C. V. Bruschi, B. Caldwell, V. Capuano, N. M. Carter, S.-K. Choi, J.-J. Codani, I. F. Connerton, N. J. Cummings, R. A. Daniel, F. Denizot, K. M. Devine, A. Düsterhöft, S. D. Ehrlich, P. T. Emmerson, K. D. Entian, J. Errington, C. Fabret, E. Ferrari, D. Foulger, C. Fritz, M. Fujita, Y. Fujita, S. Fuma, A. Galizzi, N. Galleron, S.-Y. Ghim, P. Glaser, A. Goffeau, E. J. Golightly, G. Grandi, G. Guiseppi, B. J. Guy, K. Haga, J. Haiech, C. R. Harwood, A. Hénaut, H. Hilbert, S. Hosappel, S. Hosono, M.-F. Hullo, M. Itaya, L. Jones, B. Joris, D. Karamata, Y. Kasahara, M. Klaerr-Blanchard, C. Klein, Y. Kobayashi, P. Koetter, G. Koningstein, S. Krogh, M. Kumano, K. Kurita, A. Lapidus, S. Lardinois, J. Lauber, V. Lazarevic, S.-M. Lee, A. Levine, H. Liu, S. Masuda, C. Mauel, C. Médigue, N. Medina, R. P. Mellado, M. Mizuno, D. Moestl, S. Nakai, M. Noback, D. Noone, M. O'Reilly, K. Ogawa, A. Ogiwara, B. Oudega, S. H. Park, V. Parro, T. M. Pohl, D. Portetelle, S. Porwollik, A. M. Prescott, E. Presecan, P. Pujic, B. Purnelle, G. Rapoport, M. Rey, S. Reynolds, M. Rieger, C. Rivolta, E. Rocha, B. Roche, M. Rose, Y. Sadaie, T. Sato, E. Scanlan, S. Schleich, R. Schroeter, F. Scoffone, J. Sekiguchi, A. Sekowska, S. J. Serror, P. Serror, B.-S. Shin, B. Soldo, A. Sorokin, E. Tacconi, T. Takagi, H. Takahashi, K. Takemaru, M. Takeuchi, A. Tamakoshi, T. Tanaka, P. Terpstra, A. Tognoni, V. Tosato, S. Uchiyama, M. Vandenbol, F. Vannier, A. Vassarotti, A. Viari, R. Wambutt, E. Wedler, H. Wedler, T. Weitzenecker, P. Winters, A. Wipat, H. Yamamoto, K. Yamane, K. Yasumoto, K. Yata, K. Yoshida, H.-F. Yoshikawa, E. Zumstein, H. Yoshikawa, and A. Danchin. 1997. The complete genome sequence of the Gram-positive bacterium *Bacillus subtilis*. *Nature* **390**:249–256.
- Li, X., L. Lindahl, Y. Sha, and J. M. Zengel. 1997. Analysis of the *Bacillus subtilis* S10 ribosomal protein gene cluster identifies two promoters that may be responsible for transcription of the entire 15-kilobase S10-*spc*- α cluster. *J. Bacteriol.* **179**:7046–7054.
- Lill, R., K. Cunningham, L. Brundage, K. Ito, D. Oliver, and W. Wickner. 1989. The SecA protein hydrolyzes ATP and is an essential component of the protein translocation ATPase of *E. coli*. *EMBO J.* **8**:961–966.
- Matsuyama, S.-I., J. Akimaru, and S. Mizushima. 1990. SecE-dependent overproduction of SecY in *Escherichia coli*. Evidence for interaction between two components of the secretory machinery. *FEBS Lett.* **269**:96–100.
- McNicholas, P., T. Rajapandi, and D. Oliver. 1995. SecA proteins of *Bacillus subtilis* and *Escherichia coli* possess homologous amino-terminal ATP-binding domains regulating integration into the plasma membrane. *J. Bacteriol.* **177**:7231–7237.
- McNicholas, P., R. Salavati, and D. Oliver. 1997. Dual regulation of *Escherichia coli* *secA* translation by distinct upstream elements. *J. Mol. Biol.* **265**:128–141.
- Meens, J., E. Frings, M. Klose, and R. Freudl. 1993. An outer membrane protein (OmpA) of *Escherichia coli* can be translocated across the cytoplasmic membrane of *Bacillus subtilis*. *Mol. Microbiol.* **9**:847–855.
- Miller, J. H. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Msadek, T., F. Kunst, and G. Rapoport. 1993. Two-component regulatory systems, p. 729–745. In A. L. Sonenshein, J. A. Hoch, and R. Losick (ed.), *Bacillus subtilis* and other gram-positive bacteria. American Society for Microbiology, Washington, D.C.
- Nakamura, K., A. Nakamura, H. Takamatsu, H. Yoshikawa, and K. Yamane. 1990. Cloning and characterization of a *Bacillus subtilis* gene homologous to *E. coli* *secY*. *J. Biochem.* **107**:603–607.
- Nakamura, K., H. Takamatsu, Y. Akiyama, K. Ito, and K. Yamane. 1990. Complementation of the protein transport defect of an *Escherichia coli* *secY*

- mutant (*secY24*) by *Bacillus subtilis* *secY* homologue. FEBS Lett. **273**:75–78.
29. **Nicholson, W., and P. Setlow.** 1990. Sporulation, germination and outgrowth, p. 391–450. In C. R. Harwood and S. M. Cutting (ed.), Molecular biological methods for *Bacillus*. John Wiley & Sons, Inc., New York, N.Y.
 30. **Oliver, D. B., R. J. Cabelli, and G. P. Jarosik.** 1990. SecA protein: autoregulated initiator of secretory precursor protein translocation across the *E. coli* plasma membrane. J. Bioenerg. Biomembr. **22**:311–336.
 31. **Overhoff, B., M. Klein, M. Spies, and R. Freudl.** 1991. Identification of a gene fragment which codes for the 364 amino-terminal amino acid residues of a SecA homologue from *Bacillus subtilis*: further evidence for the conservation of the protein export apparatus in gram-positive and gram-negative bacteria. Mol. Gen. Genet. **228**:417–423.
 32. **Pel, H. J., M. Rep, and L. A. Grivell.** 1992. Sequence comparison of new prokaryotic and mitochondrial members of the polypeptide chain release factor family predicts a five-domain model for release factor structure. Nucleic Acids Res. **20**:4423–4428.
 33. **Priest, F. G.** 1977. Extracellular enzyme synthesis in the genus *Bacillus*. Bacteriol. Rev. **41**:711–753.
 34. **Sadaie, Y., H. Takamatsu, K. Nakamura, and K. Yamane.** 1991. Sequencing reveals similarity of the wild-type *div⁺* gene of *Bacillus subtilis* to the *Escherichia coli* *secA* gene. Gene **98**:101–105.
 35. **Salavati, R., and D. Oliver.** 1997. Identification of elements on geneX-*secA* RNA of *Escherichia coli* required for SecA binding and *secA* auto-regulation. J. Mol. Biol. **265**:142–152.
 36. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
 37. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
 38. **Schaeffer, P.** 1969. Sporulation and the production of antibiotics, exoenzymes, and exotoxins. Bacteriol. Rev. **33**:48–71.
 39. **Schaeffer, P., J. Millet, and J. P. Aubert.** 1965. Catabolic repression of bacterial sporulation. Proc. Natl. Acad. Sci. USA **54**:704–711.
 40. **Schmidt, M. G., and D. B. Oliver.** 1989. SecA protein autogenously represses its own translation during normal protein secretion in *Escherichia coli*. J. Bacteriol. **171**:643–649.
 41. **Smith, H., W. de Vos, and S. Bron.** 1983. Transformation in *Bacillus subtilis*: properties of DNA-binding-deficient mutants. J. Bacteriol. **153**:12–20.
 42. **Strauch, M. A.** 1993. Regulation of *Bacillus subtilis* gene expression during the transition from exponential growth to stationary phase. Prog. Nucleic Acid Res. Mol. Biol. **46**:121–153.
 43. **Suh, J. W., S. A. Boylan, S. M. Thomas, K. M. Dolan, D. B. Oliver, and C. W. Price.** 1990. Isolation of a *secY* homologue from *Bacillus subtilis*: evidence for a common protein export pathway in eubacteria. Mol. Microbiol. **4**:305–314.
 44. **Suh, J. W., S. A. Boylan, S.-H. Oh, and C. W. Price.** 1996. Genetic and transcriptional organization of the *Bacillus subtilis* *spe-α* region. Gene **169**:17–23.
 45. **Swidersky, U. E., H. K. Hoffschulte, and M. Müller.** 1990. Determinants of membrane-targeting and transmembrane translocation during bacterial protein export. EMBO J. **9**:1777–1785.
 46. **Takamatsu, H., A. Nakane, Y. Sadaie, K. Nakamura, and K. Yamane.** 1994. The rapid degradation of mutant SecA protein in the *Bacillus subtilis* *secA341* (ts) mutant causes a protein translocation defect in the cell. Biosci. Biotechnol. Biochem. **58**:1845–1850.
 47. **Takamatsu, H., S. Fuma, K. Nakamura, Y. Sadaie, A. Shinkai, S. Matsuyama, S. Mizushima, and K. Yamane.** 1992. In vivo and in vitro characterization of the *secA* gene product of *Bacillus subtilis*. J. Bacteriol. **174**:4308–4316.
 48. **Tjalsma, H., M. A. Noback, S. Bron, G. Venema, K. Yamane, and J. M. van Dijl.** 1997. *Bacillus subtilis* contains four closely related type I signal peptidases with overlapping substrate specificities. Constitutive and temporally controlled expression of different *sip* genes. J. Biol. Chem. **272**:25983–25992.
 49. **Tsang, S. S., X. Yin, C. Guzzo-Arkuran, V. S. Jones, and A. J. Davison.** 1993. Loss of resolution in gel electrophoresis of RNA: a problem associated with the presence of formaldehyde gradients. BioTechniques **14**:380–381.
 50. **van der Wolk, J., M. Klose, E. Breukink, R. A. Demel, B. de Kruijff, R. Freudl, and A. J. M. Driessen.** 1993. Characterization of a *Bacillus subtilis* SecA mutant protein deficient in translocation ATPase and release from the membrane. Mol. Microbiol. **8**:31–42.
 51. **Walker, J. M.** 1994. The bicinchoninic acid (BCA) assay for protein quantification. Methods Mol. Biol. **32**:5–8.
 52. **Wickner, W., A. J. M. Driessen, and F.-U. Hartl.** 1991. The enzymology of protein translocation across the *Escherichia coli* plasma membrane. Annu. Rev. Biochem. **60**:101–124.
 53. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33**:103–119.